Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 23, SEC. F. MAY, 1945 NUMBER 3

MEASUREMENT OF LONG END GAUGES BY COMPARISON WITH PRECISION LINE STANDARDS[1]

BY CONSTANCE E. ARREGGER[2]

Abstract

The operation of comparing an end standard of length with a reference line standard is carried out by a method which is simple in principle, but which demands careful experimental technique. Textbooks give little beyond an outline of the principle, and in view of the importance of end gauges in industry, it has been considered worth while to record a description of an actual comparison. In the operation described two Johannson-type gauges, of 20 in. and 10 in. length, were compared with line standards which themselves had been compared directly with the legal Canadian standard metre.

Introduction

Purpose

As part of the work of standard maintenance in the National Research Laboratories, it became necessary to verify the lengths of two Johansson-type gauges of 20 in. and 10 in. length respectively which could then serve as reference standards. The principles employed in this comparison are simple and well known, but the lack of published data regarding the operation itself warrants a description being recorded.

Line and End Standards

Throughout the history of metrology recourse has been made to two forms of length standard. The present fundamental standards, e.g., the International Metre and the Imperial Yard, are line standards, where the length is defined by the distance between two lines engraved on the surface of a bar. Using such standards, direct comparisons can be made with subsidiary line standards, e.g., rules, tapes, etc., with the aid of length comparators and micrometer microscopes.

On the other hand, some old national length standards and certain modern gauges are end standards, in which the length is defined as the distance between two parallel end-faces of the bar or gauge. Examples are the old French Mètre des Archives and modern gauges of the Johansson type which today play such a large part in governing precision measurement in munition and other factories.

[1] *Manuscript received in original form, September 16, 1944, and, as revised, January 23, 1945.*

Contribution from the Division of Physics and Electrical Engineering, National Research Council of Canada, Ottawa, Canada. Issued as N.R.C. No. 1278.

[2] *Metrologist.*

Since an end standard of, say, one metre must, by definition, represent the same length as the fundamental one metre line standard, the metrologist is faced with the problem of comparing the one with the other. Actually the operation presents difficulties which are hard to overcome and incidentally this may be the explanation of the different values which have been obtained, by various observers, for the number of standard light wave-lengths in the metre—a difference far in excess of the expected precision of the experimental work. In all such determinations a transfer is necessary from the end standards used in the optical part of the work to the fundamental line standard.

End Gauges

The lengths of end gauges in general use vary from 0.01 in. to several feet. In workshop practice, measurements of machined components of precision instruments must, in many cases, be made to an accuracy of 10^{-4} in. or better. This accuracy cannot be achieved unless the lengths of the master gauges used by the manufacturer are known to a much greater accuracy, say 10^{-5} in. These gauges, in their turn, have to be calibrated by the metrologist whose standards must be known still more accurately—often to one or two millionths of an inch.

Interferometry provides a ready means for verifying the lengths of the shorter end gauges either by comparison with other gauges, or by direct measurement in terms of the wave-length of light of a known frequency. Further, the possibility of wringing two gauges together permits comparison of the length of one longer gauge block with the sum of the lengths of two shorter ones. This process, however, cannot be extended indefinitely without uncertainty, and for the longer gauges it is desirable to effect a direct comparison with the reference line standard.

Principle

For the purpose of comparing a long end gauge with a line standard it is the established practice to convert the end gauge virtually into a line measure by attaching to its ends auxiliary blocks which have fine transverse lines scribed on them. The distance between these lines can be compared with known lengths on a line standard in a micrometer microscope comparator. The fact that the end surfaces of Johannson-type gauge blocks are finished flat enough to permit "wringing" simplifies the procedure and avoids the necessity of clamping. Short gauge blocks serve very well for the end blocks, since their lengths can be determined independently by interferometric methods. The required length of the long gauge block can then be calculated.

Fig. 1 shows the gauge block G, to be measured, with the two auxiliary end gauges, A and B, wrung on to its ends. The fine transverse lines are ruled as nearly as possible half-way along the end gauges, dividing A and B into lengths p and q, r and s respectively. The thickness, f, of the film of liquid between the gauges which makes wringing possible must be taken into consideration. In actual practice the length of an end gauge is defined as the

distance between its ends, together with the thickness of one wringing film. This simplifies the proceedings considerably since, if the films are assumed to be of constant thickness*, the actual value of f does not have to be found.

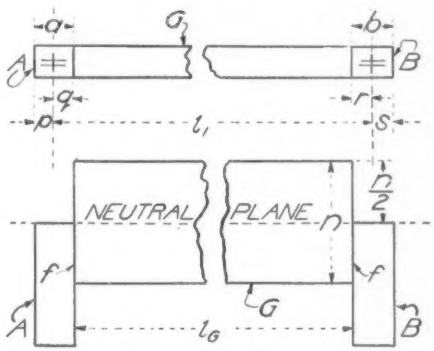

FIG. 1. *G = Long gauge to be measured; A and B = auxiliary end gauges; p, q, r, s = subdivision of gauges A and B by scribed lines; n = height of long gauge.*

From the diagram $\quad l_1 = q + f + G + f + r.$

Interchanging A and B $\quad l_2 = s + f + G + f + p.$

Adding

$$l_1 + l_2 = p + q + 2f + 2G + 2f + r + s$$
$$= (a + f) + (b + f) + 2(G + f)$$
$$\therefore G + f = \frac{l_1 + l_2}{2} - \frac{(a + f) + (b + f)}{2}.$$

Hence, if $(a + f)$ and $(b + f)$ can be found by independent means, the required value of $(G + f)$ can be obtained.

The Gauges Studied

The two gauges measured were of nominal 20 in. and 10 in. length, made of steel of cross-section $1\frac{3}{8}$ in. by $\frac{3}{8}$ in., manufactured by the Ford Motor Co. The ends of the 20 in. gauge were found to be parallel to each other within 2×10^{-5} in. and of the 10 in. within 1×10^{-5} in. when tested in the flatness and parallelism interferometer of the Metrology Section.

The End Gauges

The lengths of the auxiliary end gauges were so chosen that, when they were wrung on to the long gauges, the distance between the scribed lines was within a few microns of an integral number of centimetres, thus reducing the labour of calibrating smaller subdivisions of the line standard, in this case a one metre rule. In the case of the 20 in. gauge, by choosing gauges of length 11.5 and 12.5 mm. and scribing the lines approximately midway between

**Experiments made at the National Physical Laboratory, England, indicate that f is probably about 0.3×10^{-6} in. (3).*

the wringing surfaces, the distances l_1 and l_2 were made very close to 52 cm. For the 10 in. gauge the auxiliary end gauges chosen were 15.5 and 16.5 mm., giving a total length between the scribed lines of approximately 27 cm. The gauging surfaces of these auxiliary end gauges are finished flat and parallel to within 0.3×10^{-5} in. The upper non-gauging surface of each piece to be scribed was given a high polish by rubbing on successively finer grades of metallographic paper backed by plate glass and finally by lapping on a fine wool lap with magnesia powder and water. Fine lines were ruled on the resulting highly polished surface by means of a glass-hard·steel scriber with a finely stoned wedge-shaped cutting edge. To facilitate the setting of the micrometer microscopes always on a unique portion of each of these fine transverse lines, pairs of fine parallel lines, about 0.2 mm. apart, were drawn intersecting the original line at right angles.

In the actual operation the auxiliary end gauges were wrung on to the long gauge so that their upper inscribed surfaces were co-planar with its neutral surface when it was lying on its side with its longer cross dimension n in Fig. 1 vertical. To ensure that the scribed surface of an auxiliary end gauge was set exactly midway along the gauging surface of the gauge being measured, a combination of gauge blocks was wrung up equal to $\frac{n}{2}$ (i.e., 0.6875 in.). This was used to "square up" the position of the gauges when the long gauge was resting on a surface plate and inverted from the position it held during the comparison. To prevent lateral slipping or twisting, a steel cube on the surface plate was held against the side of the combination. It was essential that the scribed surfaces of both auxiliary end gauges should be in a horizontal plane in order that their speculum finish surfaces should reflect the field-illuminating light properly into the microscopes.

Various experiments were made in search of a suitable material for the wringing film for blocks which were to be immersed in a water-bath, and finally it was found that the blocks would remain tightly wrung after several hours' immersion if a trace of high grade kerosene were used for the film. All but the observed surfaces of the measured gauges were lacquered to prevent corrosion after the combination had been wrung up. A few crystals of potassium dichromate were dissolved in the distilled water in the comparator bath to inhibit corrosion of the uncoated surfaces.

The Standard Rules

The two rules used for reference standards were the laboratory standard metres 816 and 817. These metre-rules, made of nickel steel alloy (42% nickel), are of H-shaped cross-section. They are graduated throughout in millimetres and had been calibrated at the principal decimetre intervals by the International Bureau of Weights and Measures in 1933, and, later, had been compared with the Canadian standard metre in 1935, 1937, and 1939. This latter series of observations had shown that both 816 and 817 had shortened at a constant rate but that this rate was not the same for both

rules (2). This shortening was of the order of 1 part in 500,000 in five years. Owing to pressure of work due to war conditions it was not possible to make further observations on the rules now, but the shortening was assumed to continue at the same rates from 1939 until the present time.

When measuring the 20 in. gauge, the distance to be compared with the standard rules was approximately 52 cm. The 52 cm. interval used on each rule was that between the 28 cm. and the 80 cm. graduations. As stated previously, the value of each complete principal decimetre on the rule was known accurately but it was necessary to ascertain the value of the 2 cm. interval from the 28 to 30 cm. graduations. This was accomplished, in the comparator, by comparing, in turn, each centimetre interval between the 20 cm. and the 30 cm. graduation marks with the interval from 79 cm. to 80 cm. Since the sum of all the centimetres between 20 cm. and 30 cm. was an already certified decimetre interval, the individual values were readily computed.

For the 10 in. gauge the interval used on the rule (816) was from 23 to 50 cm. so that the subdivisions of the same decimetre could be used.

The Length Comparator

The comparison was made in the one metre line comparator (1) of the National Research Council. This consists essentially of a beam carrying two micrometer microscopes, with a carriage beneath for supporting the rules under observation. The whole apparatus is mounted on massive concrete foundations isolated from the building and carried down to bed rock. The microscopes have a magnification of about 100 diameters and a working distance of 94 mm. In each microscope one turn of its micrometer screw is approximately equivalent to 0.1 mm. at the focus of the objective. The micrometers were calibrated before the test by comparison with the standardized half-millimetre intervals on the rules 816 and 817 whose values are given in the International Bureau certificate.

In the water tank of the comparator the rule and gauge under observation are each supported, at their Airy points, on two rollers located on parallel girders. This tank rests on a carriage which can be traversed horizontally in a direction at right angles to the microscope beam. Each girder is provided with adjustments for vertical, longitudinal and transverse horizontal movements, with controls outside the tank so that the positions of the graduations on the rules can be moved in the field of view of the microscope for the purpose of focusing and aligning.

Temperature Control

Although the comparator is housed in a thermostatically controlled room, it was not considered that the temperature of rule and gauge could be kept sufficiently constant without immersion in a liquid bath. There was the risk of corrosion of the steel gauge but, as mentioned earlier, potassium dichromate

was used as an inhibitor. The water was stirred by means of a hand-operated screw propeller before and half-way through each set of readings and again at the end. Four Poulenc thermometers, subdivided to 0.1 degree, which had been calibrated in a horizontal position, were used to measure the temperature. They were held in clips, adjacent and parallel to the rule and gauge block, and agreed among themselves to 0.01° C. The thermal coefficient of expansion of the rules had been measured at the International Bureau of Weights and Measures but that of the gauge blocks was not so accurately known. It was taken to be 11×10^{-6} per 1° C.(3). Hence it was considered advisable to conduct the measurements with the temperatures as near as possible to 20° C. The variation was not greater than one degree during the period covered by the complete series of observations.

Experimental Procedure

The microscopes, clamped to the beam at the required distance apart, were adjusted for verticality by means of a sensitive level mounted across the top of a rod of the same diameter as the microscope, the rod being inserted in the bracket in place of the microscope. The position of the bracket was then adjusted by means of the screws and blocks provided for the purpose.

The field of view was illuminated by light from lamps fitted with condenser lenses and passing through Wratten green filters. This light was reflected vertically downwards by reflecting prisms in the objectives of the microscopes and then reflected upwards by the speculum finish horizontal surface of the rule or gauge block. This illumination was adjusted by aligning the lamps and filters with the windows of the reflecting prisms and by rotating the reflecting surfaces of the prisms until the path of the light incident on the rule was vertical. This was achieved by first obtaining reflection from the horizontal surface of some mercury in a dish placed in the position ordinarily occupied by the rule under observation while observing the illumination in the microscope eyepiece by means of an auxiliary lens. The reflecting prism was tilted until the exit pupil was brilliantly and uniformly illuminated. Similarly, during the tests, the rule and the gauge combination were adjusted after each "set-up" until the exit pupils were fully illuminated.

In order to ensure that the rule and inscribed gauge block surface under observation were horizontal in the length direction, the microscopes were adjusted so that both the foci were in the same horizontal plane. This was accomplished by introducing water into the tank until small particles of French chalk floating on its surface were in focus in one microscope and then adjusting the height of the other microscope until the water surface was also at its focus. The focus of the microscopes is sharp and definite and easily adjusted.

The heights of the rule and the gauge combination were adjusted to be in focus when covered with distilled water. The cover, fitted with observation windows, was put into position and the whole, after the initial adjustment, was allowed to stand for some time before observations were begun.

After stirring vigorously for 30 sec. and noting the temperature of the four thermometers the observation program consisted of setting the micrometer cross wires first on the marked portion of the scribed lines on the end gauges—noting the position of each micrometer drum, and then, after traversing the comparator carriage, setting on the corresponding graduation on the rule and noting the new position of each drum. This was repeated until 11 alternate readings had been made—the first and last readings thus being on the gauge.

The gauge combination was then turned end for end without unwringing the auxiliary end gauges and another series of readings was made. These 22 readings were used to compute a value for l_1. After interchanging the position of the auxiliary end gauges in the manner described earlier another two series of observations were made, giving a mean value for l_2. This procedure was repeated many times by each of the two observers.

Separate comparisons were made with Rules 816 and 817 and the results, obtained independently from the two rules, agreed within $\pm 1 \times 10^{-5}$ in.

Numerical Results

Fifty-two sets of observations were made using Rule 816 and 20 sets using Rule 817. After the necessary corrections for temperature variation, secular shortening of the rules, etc., the final values for the length of the 20 in. gauge were found to be 20.000163 in. when compared with Rule 816 and 20.000180 in. when compared with Rule 817. Considering the number of observations made with each rule (greater with Rule 816) the weighted mean value was computed to be 20.000168 in. The greatest deviation from the mean for any one set of the 72 sets of observations was less than 2 μ.

The length of the 10 in. gauge was found to be 10.000032 in. This comparison was made with Rule 816 only. Sixteen sets of observations were made and the deviation from the mean was within 1μ for any set of observations.

In reducing the results the length of 1 mm. was taken as 0.03937011 in.

Acknowledgment

Mr. K. Feldman of the Metrology Section assisted the writer in this work, and shared equally in the comparisons. He contributed materially also in solving the various technical difficulties that arose in the "setting-up" stage.

References

1. Field, R. H. A study of the Dominion standard yard and other standards of length. Dept. of the Interior, Canada, Topographical Survey, Bull. 60. 1929.
2. Field, R. H. Can. J. Research, A, 17 : 71-76. 1939.
3. Rolt, F. H. Gauges and fine measurements. MacMillan & Co., London. 1929.

CALIBRATION AND CHARACTERISTICS OF A SENSITIVE HOT-WIRE ANEMOMETER[1]

By T. A. Steeves[2], A. E. Chadderton[3] and W. H. Cook[4]

Abstract

A wet test meter was used to measure the air supplied to a small wind tunnel that could be adjusted to provide up-, down-, or side-draughts for the calibration of a sensitive hot-wire anemometer. The readings on the instrument used were independent of the direction of air flow and the orientation of the instrument at velocities in excess of 10 ft. per min. At lower velocities separate curves were obtained for different instrument orientations and directions of air movement. Air flows as low as 1 ft. per min. can be estimated with useful accuracy provided the direction of air movement is known.

Introduction

Air movement in cold storage rooms is an important factor in relation to spatial temperature variations and the rate of evaporation from stored goods. Within a stack of perishable products the air movement is generally less than 10 ft. per min. and may occur in almost any direction. As methods based on smoke movement are generally unsatisfactory and undesirable in stacks of edible goods, attention was turned to the use of a hot-wire anemometer.

In the course of these studies it was found that while instruments of this type could readily be standardized at zero air velocity when the sensitive portion was covered with a close fitting cover, readings as high as 5 ft. per min. were observed when the cover was removed. Obviously the air in a closed laboratory is not "still" even when precautions are taken to avoid draughts. It was therefore evident that the rotating arm method (2) was unsuitable for calibrating a sensitive instrument.

Most instruments of this type show some variation in the reading when rotated or orientated in different positions in a slow moving air stream (5). The extent of these variations depends on the air velocity and design of the instrument. Thomas (6) describes a directional heat transfer anemometer that lost its directional sensitivity at gas velocities in excess of 20 ft. per min., even at wire temperatures of 500 to 800° C. Instruments employing lower wire temperatures might reasonably become independent of directional effects at lower velocities.

At the air velocities of interest in the present application, instruments having the necessary sensitivity usually behaved normally in "up-draughts" but showed negative values in low velocity "down-draughts" such as those

[1] *Manuscript received October 27, 1944.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 130 of the Canadian Committee on Food Preservation and as N.R.C. No. 1279.

[2] *Refrigerating Engineer, Food Investigations.*

[3] *Senior Laboratory Assistant, Food Investigations.*

[4] *Director, Division of Applied Biology.*

produced by a cooling coil. Having secured a sufficiently sensitive instrument, methods had to be devised for its calibration in relation to the orientation of the instrument and the direction of air movement.

Description of Instrument

In addition to sensitivity, a satisfactory instrument for the duties indicated must be readily portable, capable of being inserted into spaces in stacked products, and independent of temperature effects. These requirements appeared to be met in a commercial instrument devised by Albrecht (1, pp. 390-397) and manufactured by R. Fuess, Berlin, Germany. Since the reported results were obtained from this instrument it will be described briefly.

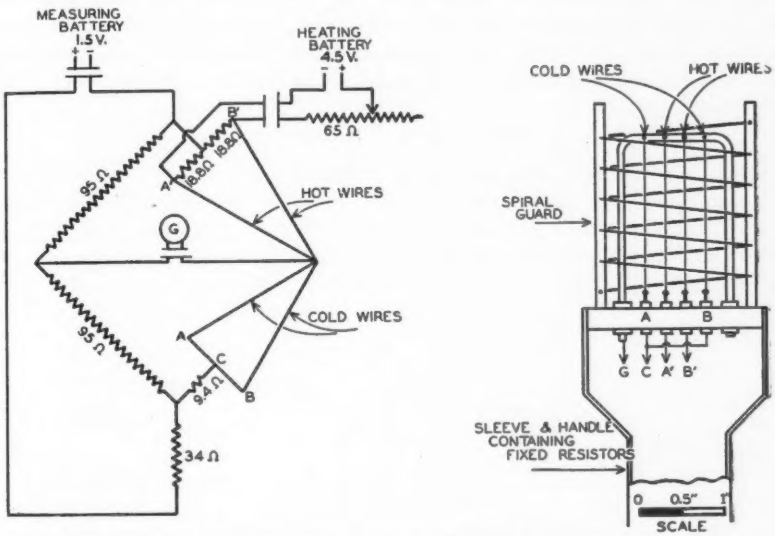

FIG. 1. *Circuit and arrangement of hot and cold wires in Albrecht anemometer.*

A drawing of the sensitive portion of the instrument and the circuit employed is shown in Fig. 1. Two sets of dry cells are required, one for heating the "hot" wires and the other for the measuring circuit. The hot and cold wires were originally made from 0.0006 in. diameter platinum, although 0.001 in. wires were later substituted, with no apparent effect on sensitivity. The use of platinum wires in both the "hot" and "cold" wire arms of the bridge compensated the instrument for changes in ambient temperature. The sensitive portion is provided with an extension handle containing the coils and extension leads to the galvanometer, batteries, and adjustable resistor. The galvanometer had two scales, one linear electrical scale (0.6 μa. per scale division) and the other an air movement scale, reading from right to left and graduated at approximately, but not exactly, logarithmic intervals.

The sensitive portion of the instrument was provided with a heavy metal cover to be used for protection when not in use and for standardization. The instruction called for the standardization to be done with the cover in place and the instrument in a vertical position, sensitive end down. In this position the heating current was adjusted by means of the resistance to bring the galvanometer to zero on the air flow scale or practically full scale electrical deflection.

The initial experiments showed that the instrument could be standardized in almost any position, although the stability of the zero adjustment was somewhat better when the recommendations were followed. Even with the cover in place, the instrument was sensitive to position; otherwise it was quite stable and returned to the zero value when returned to the vertical axis. Since air movement other than that induced by the "hot" wires must have been negligible, these observations indicated that the instrument was sensitive to orientation and this factor had to be taken into account in making the calibration.

Method of Calibration

Ower (4) describes a modification of the rotating arm method using an annular tunnel to avoid stray air currents but it was felt this would not readily lend itself to various instrument orientations. Other investigators (7) report difficulties with the rotating arm which introduces variables of too great magnitude for low velocity calibration. The present method of calibration was to deliver air at a constant rate through a precision gas meter to a small draught tunnel of known cross-section.

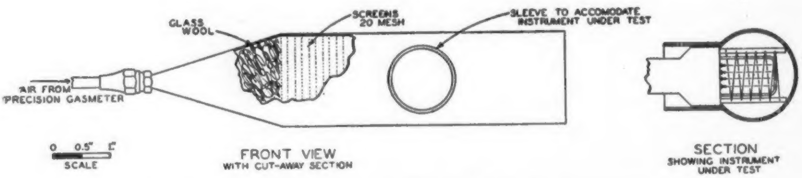

FIG. 2. *Draught-tunnel for calibrating hot-wire anemometer.*

This apparatus is shown diagrammatically in Fig. 2. A compressed air line fitted with pressure regulating devices and restrictions ahead of the gas meter insured uniform rates of flow within narrow limits. The flow rates were also checked by timing the movement of the indicating needles on the gas meter. Even minor fluctuations in flow rate were detected by the anemometer, and the observations were not recorded unless a steady condition prevailed.

Uniform air distribution throughout the tunnel was insured by filling the funnel-shaped entrance with glass wool followed by 10 layers of fine mesh screen in the cylindrical section. The tunnel exit was also covered with screen to eliminate the effect of local air currents. The side arm of the tunnel was arranged to take the instrument in any orientation. Since the tunnel itself

could be mounted in any position the air motion could be adjusted to represent side-, up-, or down-draughts. In this way almost any conceivable combination of directions of air motion or instrument orientation could be tested.

Visible examination using smokes showed that distribution of air movement within the tunnel was excellent. With the instrument and tunnel in any given position it was found that the results were remarkably reproducible from time to time.

Results

A number of calibration curves corresponding to different instrument orientations and directions of air movement were obtained, but only six curves, representing the combinations of greatest interest and practical importance, are reproduced in Fig. 3. These show the velocity in feet per minute plotted against the linear electrical scale divisions. In all instances the instrument was standardized at 80 scale divisions outside the tunnel with the protecting cover in place and the instrument in a vertical position.

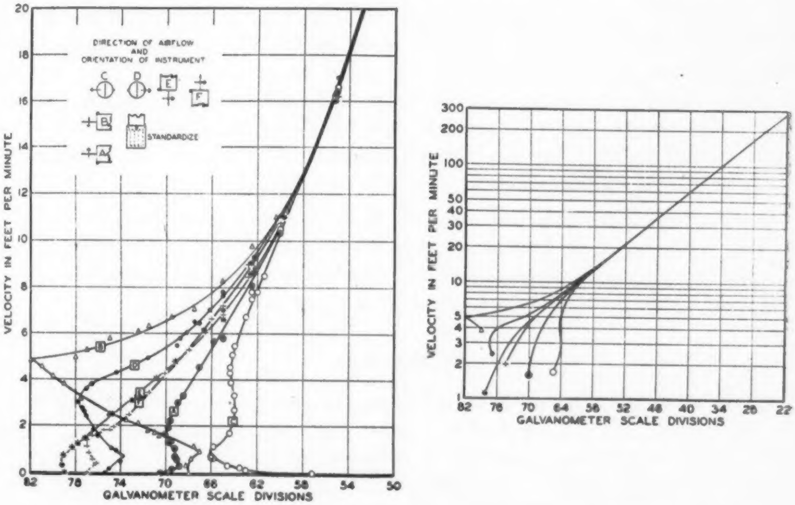

FIG. 3. *Calibration curves for various instrument orientations and directions of air flow.*

Following standardization the cover was removed and the instrument placed in the tunnel. The reading obtained at zero air velocity was dependent on the position of the instrument but was always less than the standardization value of 80. With the wires in one of the arrangements (Position *C*) the reading at zero air velocity was less than 60 scale divisions, a value corresponding to an air velocity of about 10 ft. per min. on most of the curves. These effects are attributable to the orientation of the instrument causing either an excessive cooling of the "hot" wires or excessive heating of the "cold" wires in the opposite arm of the bridge. The latter would appear to

be the more logical explanation. The difference between Positions *C* and *D* must be attributed to different resistance and temperature effects in the two parallel hot or cold wires. This explanation was confirmed in part by changing the wires in the instrument.

At low air velocities the readings may either increase or decrease, depending on the position under test. All the curves show sharp changes of direction at velocities between 0 and 1 ft. per min. This behaviour indicates interacting effects between instrument orientation and air velocity. The position and form of a particular curve are still determined primarily by the orientation of the instrument, and the initial reading at zero velocity, rather than by the rate of air movement. While the individual curves were reproducible the instrument is obviously of little value for estimating air velocities less than 1 ft. per min.

At velocities above 1 ft. per min. smooth curves were obtained when the instrument was placed vertically in a horizontal air flow. Curves representing other conditions, in particular the one obtained with a down-draught, show directional changes at velocities below 5 ft. per min. The curve for a down-draught (Position *B*) shows that the readings increase rather than decrease with increasing air velocity between 1 and 5 ft. per min. and then reverse sharply. This indicates that the effective upward air velocity induced by the "hot" wires corresponds to about 5 ft. per min., a figure in agreement with the value computed by King (3). Down-draughts of lower velocity reduce this convection, and cooling of the hot wires is therefore retarded.

At velocities above 5 ft. per min. all the curves were smooth although they differ in position at air speeds less than 10 ft. per min. Curves representing all orientations and air directions tested coincide at velocities above this value, and yield a straight line when plotted on semilog paper.

Discussion

While some attempt has been made to explain the observed behaviour of the instrument, a detailed study of these points was not undertaken. The influence of instrument orientation is doubtless peculiar to the particular design and instrument. The differential effect of up-, down-, and side-draughts may reasonably be common to all instruments of this type.

The instrument cannot be considered of any value for measuring air velocities less than 1 ft. per min. In the special case of a horizontal air stream in which the instrument can be placed in a vertical position, useful estimates can be made at velocities in excess of 1 ft. per min. In practice, low velocities in a truly horizontal direction are not likely to occur to any great extent. The more common low velocity air currents are those in which the vertical component predominates; when the velocity of such currents is less than 5 ft. per min., the present instrument is of little value unless the direction of air movement is known and the orientation of the instrument standardized. Similar precautions are necessary for measuring velocities between 5 and 10

ft. per min. but in this range estimates of varying accuracy can be made without knowing the precise direction of air movement. At velocities in excess of 10 ft. per min. the instrument is independent of directional components.

References

1. ALBRECHT, F. Handbuch der meteorologischen Instrumente und ihrer Auswertung. Springer, Berlin. 1935.
2. KING, L. V. Trans. Roy. Soc. (London) A, 214 : 373-432. 1914.
3. KING, L. V. Phil. Mag. Ser. 6. 29 : 556-577. 1915.
4. OWER, E. The measurement of air flow. Chapman & Hall, London. 1933.
5. SIMMONS, L. F. G. and BAILEY, A. Aeronautical Research Committee Reports and Memoranda No. 1019. H.M. Stationery Office, London. 1926.
6. THOMAS, J. S. G. Phil. Mag. Ser. 6. 41 : 240-258. 1921.
7. VERNON, H. M. and MANLEY, J. J. Medical Research Council Special Report Series No. 100. H.M. Stationery Office, London. 1923.

DIMETHYLGLYOXIME–COPPER TREATMENT FOR ROTPROOFING FABRICS[1]

By A. C. Neish[2], G. A. Ledingham[3], and A. G. Mackey[4]

Abstract

Copper was fixed in jute, coarse cotton, and woollen fabrics by dipping the test materials for two to five minutes in a 0.5% aqueous solution of dimethylglyoxime at 90° to 100° C., and then immersing them in 5% copper acetate at room temperature for five minutes. The amount of fixed copper was varied by altering the strength of the copper solution. The treated fabrics were odourless, remained pliable, and were dyed a khaki colour. As determined by soil burial tests this treatment proved more effective than that with copper naphthenate for rotproofing purposes.

Introduction

The rotproofing of fabrics, particularly jute and cotton, is an important problem, especially when they are to be exposed to humid tropical conditions. Copper compounds have been widely used for this purpose, and of all those reported in the literature, copper naphthenate seems to be the best (1). Although oximes have been used to precipitate copper ions from aqueous solutions, by analytical chemists, their possible use as agents for fixing copper in fabrics seems to have been overlooked. It is shown in this paper that dimethylglyoxime can be used to fix copper in fabrics, and that the resulting complex is superior to copper naphthenate in rendering the fabric resistant to rotting under the conditions of soil burial.

Experimental

Fixation of Copper in Fabrics

The dimethylglyoxime–copper treatment is a two-dip process using aqueous solutions. The dimethylglyoxime is best used as a saturated solution in water at 90° to 100° C.; immersion in a 0.5% solution for two to five minutes is satisfactory. After draining for one minute the sample is dipped in an aqueous solution of cupric acetate (or any water soluble buffered cupric salt) at room temperature for two to three minutes. The amount of copper fixed in the fabric may be controlled by varying the strength of the copper solution from 1% to saturated. Generally a 5% solution was used. It is essential, in order to avoid loss of copper, that the fabric be dried before washing. After drying, all samples were leached overnight in water at 30° C. Actually 30 min. washing is sufficient to remove all unfixed copper.

[1] *Manuscript received February 9, 1945.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 23 on the Industrial Utilization of Agricultural Wastes and Surpluses and as N.R.C. No. 1280.

[2] *Biochemist.*

[3] *Mycologist.*

[4] *Student Assistant.*

Comparable fabrics were impregnated with copper naphthenate by the standard method of dipping in a solution of the salt in Varsol (Petroleum—b.p. 147° to 198° C.). They were dried and then leached overnight in water at 30° C.

Testing Methods

The tensile strengths were measured with a Scott tester by the standard A.S.T.M. strip method.

Copper was determined in the solution obtained after ashing dry samples of fabric at 550° to 600° C. (raised to this temperature gradually from room temperature), dissolving the ash in dilute nitric acid, and evaporating the nitric acid in the presence of sulphuric acid. The solution of copper sulphate thus obtained was made up to volume, and copper determined colorimetrically in an aliquot by the ammonia method, using an Evelyn photoelectric colorimeter. This procedure was checked against the thiosulphate method.

Soil burial tests were made in greenhouse flats, using a well mixed loam. Comparative tests were run in parallel, using the same batch of soil. A temperature of 29° C. was used throughout and the soil was kept well moistened, but not waterlogged.

Results

Most of the comparative tests on the effectiveness of copper naphthenate and copper fixed by dimethylglyoxime were made using No. 8 duck. However, it was found that while the same treatment could be applied to other fabrics it was not equally effective in all cases, since the amount of copper fixed depends partly on the nature of the fabric. This is shown by the results in Table I. Jute and coarse cotton fabrics respond most favourably to this treatment though it is satisfactory with woollens also. It is difficult to fix copper in acetate rayon by this method, although viscose rayon takes it up somewhat better than fine cotton fabrics.

TABLE I

FIXATION OF COPPER BY DIMETHYLGLYOXIME TREATED FABRICS

Fabric	Copper fixed, based on dry wt. of fabric, %
Jute	1.96
No. 8 duck	1.67
Flannel blanket material	1.57
Vat dyed khaki drill	1.36
Light woollen underwear material	0.92
Light weight cotton sheeting	0.67
Nylon	0.03

The fabric was dipped in a hot (95° to 100°C.) saturated aqueous solution of dimethylglyoxime (five minutes), then in a 10% aqueous solution of copper nitrate hexahydrate buffered with 10% sodium acetate (five minutes), air-dried overnight, and thoroughly washed in distilled water to remove all water soluble copper compounds.

It was found in comparative soil burial tests (Table II) that the copper fixed by dimethylglyoxime is superior to copper naphthenate as a rotproofing agent; about one-quarter the amount of copper giving the same degree of protection in this particular experiment. The increased tensile strengths of the copper naphthenate treated samples is due to the bonding effect of this chemical on the fibres.

TABLE II

COMPARATIVE ROT RESISTANCE OF SAMPLES OF TREATED No. 8 DUCK (SOIL BURIAL)

Treatment	Fixed copper, dry wt., %		Strength, lb./in.*		
	At start	After 3 wk. burial	At start	After 1 wk. burial	After 3 wk. burial
None	Nil	Nil	223	3	Nil
Impregnated with copper naphthenate	1.75	1.70	310	292	202
	1.25	1.30	323	296	136
	0.90	0.85	310	305	95
	0.42	0.27	303	261	78
	0.28	0.05	240	236	69
Copper fixed by dimethylglyoxime	1.37	1.27	242	218	254
	1.05	0.95	221	244	243
	0.98	0.98	212	228	236
	0.78	0.63	219	222	233
	0.40	0.45	233	267	180

* *Each value is a mean of 10 determinations. Probable error of mean is about ±16 lb./in. for the 0.01 level of significance in case of samples at start.*

A further comparison was made between the two copper treatments using soil in which copper tolerant moulds were even more abundant than in the soil used in the above experiment. The results (see Fig. 1) also show that the dimethylglyoxime–copper treatment is superior to copper naphthenate treatment. The rapid loss of fixed copper from fabrics in this soil appears to be caused by a species of *Penicillium*, which grows in scattered colonies over the surface of the material, and removes copper at these points.

A sample of jute containing 1.5% copper fixed by dimethylglyoxime showed no loss in tensile strength after five weeks' burial in soil in which an untreated sample was completely rotted in eight days.

In addition to increased protection against rotting per unit copper the dimethylglyoxime–copper treatment has other advantages that should be mentioned. The most important is that treated fabrics remain pliable, whereas equivalent copper as naphthenate stiffens them. Furthermore, the new treatment leaves fabrics odourless and imparts a pleasing khaki colour that is decidedly preferable to the blue colour of copper naphthenate for military purposes.

Tests have shown that the dimethylglyoxime alone has no fungicidal properties. Loss in strength of treated fabrics was correlated with loss in copper content. Nickel–dimethylglyoxime treatments were tested also, but proved to be definitely inferior.

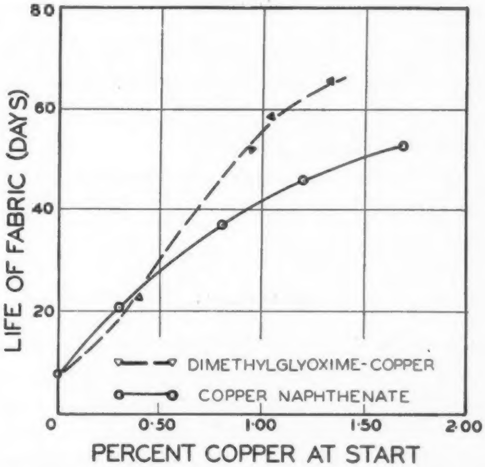

FIG. 1. *Rot resistance of treated duck. Samples of No. 8 duck were subjected to copper naphthenate or dimethylglyoxime–copper treatments and then buried in soil. The life of the fabric is the time required for the tensile strength to fall to about 2% of its original value.*

Discussion

In addition to dimethylglyoxime, there are other oximes that may be suitable for this type of treatment, since they are known to form water insoluble complexes with copper. These include, benzoin oxime, salicylaldoxime, furoin oxime, and furil dioxime. If a demand were created for any of these they could be produced to sell at a much lower price than that quoted at present. Even if dimethylglyoxime were purchased as an analytical reagent, at present prices, the cost of the above treatment is only about 10% of the cost of the untreated fabric.

Dimethylglyoxime can be readily produced from 2,3-butanediol, and furoin and furil oximes from furfural; thus it is possible to make these chemicals from agricultural wastes and surpluses.

Acknowledgments

The authors are indebted to W. I. Illman for assistance with the soil burial tests, and to the textile laboratories, Division of Chemistry, for help with the determination of tensile strengths.

Reference

1. MARSH, P. B., GREATHOUSE, G. A., BOLLENBACHER, K., and BUTLER, M. L. Ind. Eng. Chem. 36 : 176-181. 1944.

STUDIES ON THE PROCESSING OF WHEAT GERM[1]

BY C. V. LUSENA[2] AND W. D. MCFARLANE[3]

Abstract

Processes are described for the controlled treatment of wheat germ with steam or with ethylene dichloride vapours followed by steam. The products are highly palatable and have excellent keeping and baking qualities. The improved quality is associated with the inactivation of proteolytic enzymes, low moisture content, and the removal of odoriferous substances. The treatments destroy a considerable part of the glutathione but do not affect the vitamin E and thiamin content or promote rancidity.

To facilitate these studies on processing, a new procedure has been developed for measuring the proteolytic activity of wheat germ, and previous methods have been adapted for the determination of the lipoxidase activity and glutathione content of wheat germ. No relationship is found between the development of off-flavours in wheat germ during storage and the formation of peroxides or lipoxidase activity.

Introduction

The primary aim of this investigation was to improve the palatability and keeping quality of wheat germ so as to make it more acceptable as a human food. Wheat germ is a highly nutritive product, being a rich source of the B vitamins, high quality protein, fat, and vitamin E. However, cereal germ, being a living system from which a whole plant can develop, possesses an intricate system of enzymes and activators that are responsible for some of the difficulties attending the use of this product as food; it quickly develops "off-flavours" in storage and it liquefies the dough in bread-making. It would appear that both problems could be overcome by inactivating the enzymes, but this would have to be accomplished by a treatment that has no deleterious effect on palatability or nutritive value.

Sumner (11) reported the presence of lipoxidase in wheat germ; therefore, fat oxidation may be a cause of deterioration during storage. "Tonik" Wheat Germ,* which is the fat-free residue obtained by extraction with ethylene dichloride, has good keeping quality. However, Pearce (10) did not find an increase in peroxides in whole wheat germ during storage, but obtained results that indicate that protein hydrolysis is chiefly responsible for the spoilage. Apparently, therefore, the processing of "Tonik" Wheat Germ with ethylene dichloride also inhibits protein decomposition.

Highly toasted wheat germ is said to have improved keeping and baking qualities (7) but toasting destroys the characteristic flavour and odour of fresh wheat germ. However, the results obtained by this treatment suggest that a stable product might be procured, without adversely affecting the

[1] *Manuscript received in original form October 24, 1944, and as revised, January 2, 1945.*
Contribution from the Department of Chemistry, Faculty of Agriculture (McGill University), Macdonald College, Que. Macdonald College Journal Series No. 195.

[2] *Research Assistant in Chemistry.*
[3] *Professor of Chemistry.*

* *A product of Viobin (Canada) Ltd., Montreal.*

flavour and odour, by a less drastic heat treatment under controlled conditions. The development of such a process is described in this paper and data are presented on the effect of the treatment on the enzymic activity of the product in relation to its keeping and baking qualities.

Analytical Methods

I. Lipoxidase Activity

Sumner's method (11) was adapted to the determination of the lipoxidase activity of wheat germ, the lipoxidase extract being prepared as follows:

Finely ground wheat germ (2.5 gm.) was weighed into a 250 ml. Erlenmeyer flask, 100 ml. of distilled water was added, and the flask shaken for 30 min. in a mechanical shaker. A portion of the material was centrifuged for 10 min. at 2000 r.p.m. and the clear supernatant enzyme extract was separated by decantation.

The test was carried out according to the directions given by Sumner, using a freshly prepared solution of linoleic acid as the substrate and employing a Coleman Spectrophotometer set at 455 mμ for the final measurement of the red colour. The ferric ion concentration in micrograms per millilitre in the test and blank solutions was obtained by reference to a calibration curve previously prepared from data obtained by applying the thiocyanate reaction, under the exact conditions of the test, to standard solutions of ferric chloride containing 0.5–8 μgm. Fe^{+++} per ml.

Since the calibration curve is logarithmic, the sensitivity of the method decreases at high colour intensities. For this reason no readings were taken at concentrations greater than 4 μgm. Fe^{+++} per ml. Taking into consideration (*a*) the range of accuracy of the calibration curve, (*b*) maximum values, and (*c*) the straight-line portion of the curve obtained by plotting peroxide formation against time for two samples of wheat germ, the optimum reaction time was found to be five minutes. The ferric thiocyanate colour was developed under red light, since bright daylight or electric light increases the intensity of the colour. The wheat germ could be ground without sand satisfactorily in a mortar. When acid-washed sand was used, the blank contained an excessive amount of iron. Filtering the extract reduced the lipoxidase content by as much as 50%.

II. Proteolytic Activity

To measure proteolytic activity, Anson (1) utilized the blue colour given with the phenol reagent and expressed the results in milliequivalents of tyrosine. This reaction was employed as the basis of the following method, which we have developed for determining the proteolytic activity of wheat germ and in which the whole wheat germ is used as the substrate and the source of the enzyme.

Into each of four Erlenmeyer flasks was weighed 0.25 gm. of finely ground wheat germ. Two of the flasks were used as blanks and to these was added 20 ml. of a 10% solution of trichloroacetic acid. To all of the flasks was added

20 ml. of citrate buffer pH 4.5 (prepared by mixing 70 ml. of a solution containing 21 gm. of citric acid and 8 gm. of sodium hydroxide with 30 ml. of 0.1 N hydrochloric acid), and 100 ml. of distilled water. After incubating at 37° C. for six hours, 20 ml. of 10% trichloroacetic acid was added to the two test flasks, and, after mixing, the solutions were filtered through Whatman 42 paper. One-millilitre aliquots of each filtrate were placed in large test-tubes, and 20 ml. of water, 1 ml. of 3.85 N sodium hydroxide and 1 ml. of phenol reagent (5) were added to each. After standing 10 min. the colour intensity was measured in a Coleman Spectrophotometer set at 650 mμ.

The tyrosine equivalent of the blue colour was obtained by reference to a calibration curve previously prepared from data obtained by applying the "phenol reaction", under the exact conditions of the test as described above, to standard solutions containing 40 to 160 μgm. of tyrosine per ml.

The following equation was used to calculate the units of proteolytic activity as milliequivalents of tyrosine per kilogram wheat germ:

$$\frac{(T - B) \times V}{E \times W} = (T - B) \times 3.1$$

where T = Tyrosine in test solution in μgm. per ml.
B = Tyrosine in blank solution in μgm. per ml.
V = Volume of solution (140 ml.)
W = Weight of sample (0.25 gm.)
E = Equivalent weight of tyrosine (181).

It was realized that not all of the blue colour developed in the hydrolysate was due to tyrosine; however, tyrosine was used as a convenient standard. The calibration curve was not logarithmic and the most sensitive readings were obtained in a range of 10 to 80 μgm. of tyrosine per ml. Less than 0.2 gm. or more than 0.5 gm. of wheat germ in 140 ml. digestion mixture gave readings beyond the limits of the accurate range of the calibration curve.

Since it is well established that the proteolytic enzyme of wheat germ is of the papain type, the activity was tested over a range of pH 3.5 to 6 and the optimum was found to be pH 4.5. The optimum digestion time and temperature, compatible with the accurate range of the calibration curve, were found to be six hours and 37° C. Apparently wheat germ already contains an optimum amount of activators, since the addition of glutathione did not accelerate proteolysis. The addition of sodium cyanide slightly reduced the activity, thus indicating some effect of heavy metals.

III. Glutathione

The determination was carried out by the method of Binet and Weller (2, 3, 12), which is based on the quantitative precipitation of glutathione by cadmium lactate and determination of the glutathione in the precipitate by iodimetry. The glutathione extract was prepared by triturating 1 gm. of wheat germ in a mortar with 5 ml. of 95% ethanol and extracting with three 10 ml. portions of 10% trichloroacetic acid. Quantitative precipitation of

glutathione with cadmium lactate was confirmed with solutions of pure glutathione and by the recovery of known amounts of glutathione added to extracts of wheat germ. Cysteine did not interfere.

IV. Fat-peroxides

The formation of peroxides in wheat germ during storage was followed by means of the method of Chapman and McFarlane (4), employing 0.2 gm. samples.

Experimental

The inactivation or destruction of the enzymes in wheat germ is chiefly a problem of controlling the oxidation or denaturation of the protein without affecting the quality of the product. In a preliminary experiment, fresh wheat germ was subjected to various heat treatments in a hot-air oven, as indicated in Table I. The samples heated for 12 hr. at 100° C. in open containers were dry and had a good colour and pleasant taste and odour, whereas samples subjected to the same treatment but in closed flasks (not recorded in Table I) had a dark brown colour and a disagreeable taste and odour.

TABLE I

EFFECT OF VARIOUS HEAT TREATMENTS AND SUBSEQUENT STORAGE CONDITIONS

Sample No.	Treatment	Storage atmosphere	Moisture, %	Lipoxidase activity	Proteolytic activity	Peroxide formation during storage, m.e. per kgm.			
				Units per kgm.		Zero	1	3	5*
1	Control—untreated	Air	11.1	537	42	Nil	Nil	0.4	16.0
2	Control—untreated	Nitrogen	11.1	537	42	Nil	Nil	0.3	14.3
3	1.5 hr. at 100° C. in air—closed system	Air	10.3	Nil	Nil	Nil	0.2	1.2	20.3
4	1.5 hr. at 100° C. in air—closed system	Nitrogen	9.6	Nil	20	Nil	0.1	1.5	23.4
5	1.5 hr. at 100° C. in nitrogen—closed system	Air	8.6	Nil	23	Nil	Nil	0.9	10.0
6	1.5 hr. at 100° C. in nitrogen—closed system	Nitrogen	9.7	Nil	16	Nil	Nil	0.9	5.3
7	12 hr. at 100° C. in air—open system	Air	0.9	Nil	35	1.0	20.0	39.0	43.0
8	12 hr. at 100° C. in air—open system	Nitrogen	0.9	Nil	35	1.0	10.1	54.7	60.0

* *Duration of storage at 37° C. in weeks.*

Ten samples from each of the eight treatments were air- or nitrogen-packed in laminated metal-foil envelopes*, heat-sealed, and stored at 37° C. Packages were opened at weekly intervals and the peroxide content of the samples determined. The experiment was discontinued after five weeks' storage when all the samples had developed strong off-flavours. The results in Table I indicate that:

* *Supplied by Reynolds Metal Co., Richmond, Virginia.*

(*a*) All the heat treatments destroyed lipoxidase and there was little or no relation between lipoxidase activity and the subsequent peroxide formation during storage. The control samples, with the enzyme active, showed a smaller increase in peroxides than the heat-treated samples.

(*b*) Heating under nitrogen was less conducive to peroxide formation than heating in air (compare Treatments 3 and 4 with 5 and 6). Prolonged heating in air favoured the subsequent formation of peroxides during storage (Treatments 7 and 8). There was no beneficial effect from nitrogen-packing.

(*c*) The proteolytic activity of all the treated samples was reduced and the effect was most marked in the samples with the highest moisture content.

Rancidity in wheat-germ was apparent by smell and taste when the peroxide value had increased to about 20 m.e. per kgm. However, off-flavours were least pronounced in Samples 7 and 8, thus indicating that the peroxide value was not a reliable criterion of keeping quality. Apparently, the fat was not the main source of these off-flavours and moisture played an important part in their development.

Successive Moist and Dry Heat-treatments

Since the presence of moisture facilitated the destruction of proteolytic enzymes, and, as a sample with a low moisture content showed less tendency to develop off-flavours in storage, experiments were conducted to determine the effect of treating the sample first with moist heat and then with dry heat.

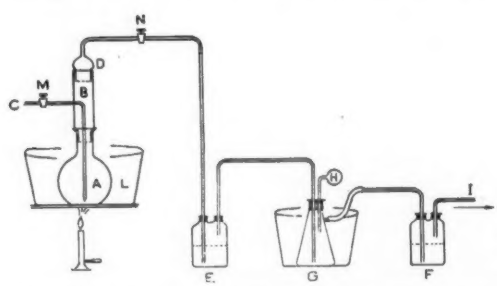

FIG. 1. *Apparatus for the successive moist and dry heat-treatments of wheat germ.*

The apparatus employed is illustrated in Fig. 1 and was operated as follows:

About 400 gm. of wheat germ was placed in the one-litre round-bottomed flask, *A*, which was connected through *C* to a cylinder of nitrogen and through *D* to two calcium chloride traps, *E*, *F*; an ice trap, *G*; a manometer, *H*, and a "Hyvac" pump, *I*. The flask was evacuated and filled with nitrogen three times; stopcocks *M* and *N* were closed; and the flask was heated in a boiling water-bath. After 1.5 hr. of moist heat-treatment, stopcocks *M* and *N* were opened and the dry heat-treatment was applied in an oxygen-free atmosphere by slowly passing nitrogen through the wheat germ (still heated by the boiling water-bath) for 1.5 hr. The flask was evacuated at the begin-

ning and towards the end of this period, to facilitate the removal of volatile substances. The water-bath was removed and the wheat germ cooled while nitrogen was passed through it for 30 min.

The times given for the duration of the moist heat and dry heat treatments were arrived at by a series of experiments, and are the minimum required to destroy lipolytic and proteolytic activity and to assure the best keeping quality. Samples of the treated product were air- or nitrogen-packed in heat-sealing wax and resin-coated Cellophane envelopes* and stored at 55° C. The material kept fresh and palatable for at least one month, whereas untreated control samples were definitely off-flavour. It is noteworthy that the peroxide values of the control and treated samples, after one-month's storage, were of the same order, i.e., 0.2 to 1.0 m.e. per kgm. Packing in nitrogen or in air made little difference in the keeping quality of the product.

Treatment with Ethylene Dichloride Vapours

The stability of "Tonik" Wheat Germ has already been referred to and it was suggested that the solvent used in its preparation (ethylene dichloride) had a preserving action on the non-fat constituents of the germ. A sample of "Tonik" Wheat Germ was found to have a proteolytic activity of 13 units per kgm., as compared to 30 units for raw wheat germ. The effect of ethylene dichloride vapours on the lipolytic and proteolytic activity of wheat germ was next investigated as follows:

The apparatus was similar to that shown in Fig. 1 but a condenser with vacuum adapter was attached to flask *A* through *D* and a T-shaped connecting tube with stopcocks was attached at *C*. One arm of the connecting tube led to a distilling flask in which the ethylene dichloride was boiled and the other to a nitrogen or carbon dioxide cylinder. The vapour from one litre of ethylene dichloride was passed through 400 gm. of wheat germ that was held at 15 to 20° C. above the boiling point of the solvent by heating the flask on a water-bath. Carbon dioxide was then passed through for two hours and finally the wheat germ was allowed to cool under reduced pressure.

This sample had a pleasant nutty flavour and retained its freshness during three and one-half months' storage at 55° C. The only difficulty encountered in processing was to remove the last traces of solvent and this necessitated the prolonged treatment with carbon dioxide. It was found that the residual solvent could be most effectively removed by passing steam through the wheat germ. The ethylene dichloride and steam treatments were combined in the following process:

A 250 gm. sample of wheat germ was placed in the flask and held at 100° C. while the vapours from 600 ml. of ethylene dichloride passed through in 1.5 hr. The wheat germ was next deodorized by passing steam through for one hour under a vacuum of about 0.5 cm. of mercury. Finally the wheat germ was dried by holding it under reduced pressure for another hour while the flask was heated on the boiling water-bath.

* *Supplied by Cellophane Division, Canadian Industries Ltd., Montreal.*

The product contained 4.7% of moisture and had no lipolytic or proteolytic activity. It was extremely palatable and retained its freshness after eight months' storage in Cellophane envelopes at 37° C., whereas the untreated control sample was off-flavour at the end of one month. It was later found that a comparable treatment with steam alone was just as effective and there appeared to be no special advantage to using ethylene dichloride. The treatment with ethylene dichloride will be referred to below in connection with the activation of the proteolytic enzymes of wheat germ by glutathione.

Steam Treatment in Metal Equipment

The chief difficulty in the process described above was to obtain a uniform treatment. Equipment was therefore designed so that the wheat germ could be agitated during the steam treatment and subsequent drying in an inert atmosphere. The apparatus consisted of a hollow brass cylinder coated on the inside with "Lithcote", and held horizontally on a revolving shaft which projected half-way into the cylinder and through which the steam or inert gas was also introduced. The cylinder was fitted with a screw-top lid with outlet-tube and was steam-jacketed. A typical processing was carried out as follows:

Wheat germ (200 gm.) was placed in the chamber, which was set in motion at about 40 r.p.m. The chamber was heated to 97° C. in 10 min. by admitting steam into the jacket. Steam, generated at constant temperature, was passed through the chamber so that the inside temperature was raised to 110° C. in two minutes. The steam treatment was continued for 30 min. at 110° C. and then nitrogen was passed through the chamber, held at 100° C., for 40 min. Finally the chamber was cooled to room temperature in seven minutes by passing cold water through the steam jacket.

The products were comparable in palatability and keeping quality to those prepared in the all-glass apparatus by a similar process.

Baking Trials with Treated Wheat Germ

Some of the samples processed in the metal equipment by the method last referred to were used in baking tea biscuits. The treated samples and the control "raw" germ were tested at different levels. The appearance and taste of the biscuits baked with flour containing up to 20% of treated wheat germ were as good as, if not better than, those of the biscuits prepared with straight flour, but at the 20% level the biscuits were slightly "heavy". The wheat germ improved the palatability, probably because of the extra fat it contributed. Untreated germ imparted some of its "raw" taste to the biscuits.

Samples of treated germ were compared with "raw" germ in baking tests that were conducted as follows:

The composition of the dough was: 380 gm. of "Buffalo" flour, 19 gm. of wheat germ (5% of the flour), 11.4 gm. of yeast, 11.4 gm. of sugar, 6.6 gm. of salt, and 200 ml. of water. The yeast was dissolved in 100 ml. of water and the salt and sugar were dissolved in the remainder of the water. The ingredients were mixed in a Hobart mixer, fitted with a cake paddle. Loaf

volume, crumb colour, texture, and comments on the handling qualities were recorded.

The results of a typical experiment are shown in Table II. The loaves containing treated germ were not as brown on the outside and showed better symmetry than those containing untreated germ. There was no difference

TABLE II

EFFECT ON LOAF VOLUME AND CRUMB COLOUR OF TREATED AND RAW WHEAT GERM

	Proteolytic activity of germ, units per kgm.	Loaf volume*, ml.	Crumb colour (relative order)
1. Flour alone	—	2650	—
2. 95% Flour alone (calculated)	—	2510	—
3. Flour + 5% raw germ	29	2500	4
4. Flour + 5% treated germ			
15 min. *S* and 40 min. *N*	13	2500	3
20 min. *S* and 30 min. *N*	9	2500	3
30 min. *S* and 45 min. *N*	Trace	2575	2
60 min. *S* and 35 min. *N*	Nil	2600	2
25 min. *S* and 45 min. *A*	Nil	2600	1

S = steam treatment at 105 to 110° C.; N = drying at 100° C. in current of nitrogen; A = drying at 100° C. in air.

* *The necessary difference for a significant variation in loaf volume is 75 ml.*

in the texture of the loaves, with the exception of those containing "raw" germ, which were very coarse and open. There was also no difference in the handling quality of the doughs containing "treated" germ and no excessive softening was noted. It may be concluded from the results in Table II, that (*a*) loaf volume was increased by treated germ when its proteolytic activity was abolished, and (*b*) there was a marked correlation between baking quality and proteolytic deactivation. Geddes (6) has reported that germ added to fifth middlings flour markedly reduced its baking quality, and that heating the germ before admixture reduced the deleterious effect. Oxidation of certain germ constituents, presumably the phosphatides, was suggested as the primary change involved in such improvement.

Samples of raw and treated germ that had been stored for eight months at 37° C. in Cellophane envelopes were also subjected to baking tests as described above. The harmful effect of raw germ on loaf volume and texture was no longer apparent, but, of course, the germ had now spoiled in storage. Excellent loaves were obtained with germ that had been treated with ethylene dichloride and steam.

Effect of Treatment on the Composition of Wheat Germ

Vitamin E determinations (9) on the crude fat extracted from raw and treated germ that had been stored for eight months at 37° C. showed that no

loss resulted either from the treatment or during the subsequent storage. The thiamin content of the samples, as determined by the thiochrome method, was also unimpaired. It may be assumed, on the basis of the observations of Hove and Harrel (8), that the biological value of the germ proteins was also unaffected.

The effect of treatment on the glutathione content of wheat germ was also investigated in relation to the activation of proteases. The results presented in Table III indicate that the treatments reduced the total glutathione content by 50% and the reduced form by 25%, with complete destruction of the oxidized form. Apparently the treatment carried the oxidation of glutathione beyond the dithio-form, possibly causing complete decomposition.

TABLE III

THE GLUTATHIONE CONTENT OF RAW AND TREATED WHEAT GERM

Sample	Glutathione, mgm./100 gm.		
	Total	Reduced	Oxidized (by difference)
1. Raw germ	147.4	102.6	44.8
2. Treated germ			
A. 30 min. *S* and 1 hr. *D*	79.8	80.5	Nil
B. 15 min. *E* and 15 min. *S* and 1 hr. *D*	76.7	77.2	Nil

S = Steam at 105 to 110° C., D = drying in air at 100° C., E = ethylene dichloride vapour at 95 to 100° C.

By adding glutathione to the digest of treated wheat germ, as prepared for the determination of proteolytic activity, it should be possible to ascertain whether the reduction in activity was directly due to the denaturation of the

TABLE IV

THE EFFECT OF GLUTATHIONE AND SODIUM CYANIDE ON THE PROTEOLYTIC ACTIVITY OF RAW AND TREATED WHEAT GERM

	Proteolytic activity, units per kgm.		
	Raw germ	Treated germ	
		(*a*) Ethylene dichloride	(*b*) Steam
No addition	31	6	Nil
0.5 Mgm. glutathione*	31	22	Nil
1.0 Mgm. glutathione*	31	28	Nil
0.25 Mgm. sodium cyanide*	28	28	Nil

(a) 15 Min. ethylene dichloride, 15 min. steam, and one hour air.

(b) 30 Min. steam and one hour air.

* *Amount added to the total extract from 0.25 gm. of wheat germ.*

protein-enzyme or, indirectly, to the destruction of glutathione. The results of such an experiment are given in Table IV.

Apparently the steam treatment denatured the proteolytic enzymes so that they were not reactivated by glutathione or cyanide. On the other hand, the proteolytic activity of the ethylene-dichloride–treated sample was almost completely restored. Activation by cyanide indicates that the papain-like enzyme was changed by the treatment to the reduced inactive form. The effect of cyanide on the proteolytic activity of raw germ might be due to the poisoning of some related enzyme system containing heavy metal.

Acknowledgments

The authors are indebted to Dr. W. E. Parker and Mr. H. Johannson of the Ogilvie Flour Mills Co., Ltd., Montreal, for suggestions during the course of this investigation, and for carrying out the baking tests. Their thanks are also due to R. F. Walsh Co. Ltd., Montreal, for "Lithcoting" the metal equipment.

References

1. Anson, M. L. J. Gen. Physiol. 20 : 561-563. 1936.
2. Binet, L. and Weller, G. Bull. soc. chim. biol. 16 : 1284-1296. 1934.
3. Binet, L. and Weller, G. Compt. rend. soc. biol. 119 : 939-941. 1935.
4. Chapman, R. A. and McFarlane, W. D. Can. J. Research, B, 21 : 133-139. 1943.
5. Folin, O. and Ciocalteu, V. J. Biol. Chem. 73 : 627-650. 1927.
6. Geddes, W. F. Can. J. Research, 2 : 195-213. 1930.
7. Hertwig, R. Cereal Chem. 8 : 229-231. 1931.
8. Hove, E. L. and Harrel, C. G. Cereal Chem. 20 : 141-148. 1943.
9. Parker, W. E. and McFarlane, W. D. Can. J. Research, B, 18 : 405-409. 1940.
10. Pearce, J. A. Can. J. Research, C, 21 : 57-65. 1943.
11. Sumner, R. J. Ind. Eng. Chem., Anal. Ed. 15 : 14-15. 1943.
12. Weller, G. Ann. fermentations, 1 : 108-114. 1935.